9 THIN LAYER CHROMATOGRAPHY

9.1 Introduction:

9.1.1 Thin layer chromatography (TLC) is a useful method for screening, separation and preliminary identification. Both the approximate concentration of the sample and the number of components contained in the sample can be ascertained by TLC. TLC can provide valuable information before proceeding on to instrumental tests. Clues as to the chemical structure of an analyte can be obtained by noting the distance traveled in different solvent systems and noting the reactions to a variety of chemical sprays.

9.1.2 The specificity of TLC is greatly increased by using multiple solvent systems of different polarities or pH. Because many drug compounds (or other organic compounds) have similar Rf values in any one solvent system, at least two solvent systems should be used, except for marijuana and hashish oil.

9.1.3 Specific solvent systems and developing sprays utilized in casework will be denoted in the analytical case notes. Positive TLC results may be recorded in the analytical notes by the use of a plus (+), a plus circled (⊕) or an abbreviation (e.g., pos) along with the standard used in the comparison. A result is considered positive when the distance traveled and the reaction with the visualization methods compare favorably with a standard. Negative reactions may be recorded in a similar fashion; standards used for which negative results are observed need not be documented.

9.2 Materials:

9.2.1 Solvent tank - Any covered glass container with a level bottom can be used. Rectangular tanks are most common. The developing solvent should be at a depth of approximately 0.5 cm to maintain constant contact with the stationary phase throughout the analysis. Filter paper or some other suitable absorbent paper should line the back inside wall of the tank at a height greater than the plate being used when it is required to maintain an atmosphere saturated with solvent vapor. This can provide for better migration and more consistent results. Care should be taken to maintain this atmosphere. The absorbent paper is not required for marijuana analysis.

9.2.2 Thin layer plates - silica gel (250μm) coated glass plates with a fluorescent indicator, or equivalent (Most drug compounds quench fluorescence when visualized under short wave UV light)

9.2.3 Capillary tubes or Micropipettes

9.2.4 Long wave/short wave UV light source

9.2.5 Solvent baths

9.2.6 Visualization reagents

9.3 Methods:

9.3.1 The sample to be tested is dissolved in $CHCl_3$, MeOH or other suitable solvent. The solution is drawn up into a capillary tube and 1-10 μl (depending on concentration) is spotted on a dry plate approximately 0.5 - 1 cm from the bottom, making sure that the spot is above the solvent level in the developing tank. The spot size should be kept to a minimum as its diameter will increase while the compound migrates up the plate during development. Heavy concentrations should be avoided as this causes streaking and tailing.

9.3.2 A standard is spotted beside the sample(s) for comparison. Care should be taken that the standard and sample(s) are approximately the same concentration. Unequal concentrations frequently result in unequal rates of advance. This can easily be checked by visualizing the plate under UV light before development.

9.3.3 The plate is placed in the tank and allowed to develop until the solvent reaches the top. The plate is then removed, dried, inspected under UV light, and/or sprayed with the appropriate visualizing reagent. Do not allow the plate to stand in the solvent after development is complete as this will cause a gradual diffusion of the compound.

9.3.4 A blank should also be spotted on a periodic basis as a convenient means of checking solvent cleanliness. When blanks are run, the results of the blank must be recorded in the case notes. This may be done by using a check mark (√) or "ok" or "-" to record that the results of the blank were acceptable (e.g., Blk √).

9.4 TLC Baths:

- TLC1: $CHCl_3/CH_3OH$ (9:1)(v/v) – general drug screening (9.8.1)
- TLC2: Ammonia washed $CHCl_3/CH_3OH$ - (18:1) (v/v) – general drug screening (9.8.2)
- TLC3: T-1 Methanol/NH_4OH (100:1.5) (v/v) – general drug screening (9.8.1)
- TLC4: 8% Diethylamine in Toluene (v/v) – marijuana / general drug screening (9.8.3)
- TLC5: 4% Diethylamine in Toluene (v/v) – marijuana – general drug screening (9.8.3)
- TLC6: Chloroform/Ethyl Acetate (80:20) (v/v) – steroids (9.8.5)
- TLC7: Isopropyl Ether – barbiturates (9.8.8)
- TLC8: Acetone /$CHCl_3$ (2:1)(v/v) – LSD/LAMPA (9.8.2)
- TLC9: Cyclohexane/Toluene/Diethylamine (75:15:10) (v/v) – MDMA/methamphetamine (9.8.1)
- TLC10: Ethyl Acetate – GHB, GBL, 1,4-butanediol
- TLC11: Deionized water - vitamins
- TLC12: Acetone – amphetamines and other basic drugs (9.8.4)
- TLC13: Ethyl acetate/acetone/ammonium hydroxide (25:5:1) (v/v) – ephedrine/psuedoephedrine

9.5 Visualization Reagents:

9.5.1 All visualization sprays must be used in a fume hood.

9.5.2 Ceric Sulfate (9.8.10)

9.5.2.1 Used as an overspray to intensify the reaction with iodoplatinate, especially for caffeine

9.5.2.2 Recipe: 5 g $Ce(SO_4)_2$ in 500 mL H_2O and 14 mL H_2SO_4.

9.5.3 Diphenylcarbazone (9.8.6)

9.5.3.1 Used as an overspray with mercuric sulfate for barbiturates. Can also be freshly mixed 50/50 with the mercuric sulfate reagent.

9.5.3.2 Recipe: 19 mg diphenylcarbazone in 200 mL (50% acetone/water).

9.5.4 Dragendorff (9.8.1)

9.5.4.1 General spray which visualizes alkaloids and other nitrogen containing compounds, including methamphetamine and diazepam.

9.5.4.2 Recipe: 1.3 g of bismuth subnitrate in 60 mL water with 15 mL glacial acetic acid. Add this to 12 g potassium iodide in 30 mL H_2O. Dilute with 100 mL of H_2O and 25 mL glacial acetic acid.

9.5.5 Ehrlich's or p-Dimethylaminobenzaldehyde (p-DMAB) (9.8.7)

9.5.5.1 Visualizes LSD and psilocybin, reacts with indole nucleus of alkaloids. Plate may be heated after spraying to increase intensity of color.

9.5.5.2 Recipe: 2 g of p-DMAB in 50 mL 95% ethanol and 50 mL 37% HCl.

9.5.6 Fast Blue B (9.8.1) or Fast Blue BB (9.8.9)

9.5.6.1 Visualizes the three major cannabinoids in marijuana. They migrate and develop in the following order:

- Top spot - Cannabidiol – orange
- Middle spot - Tetrahydrocannabinol (Δ^9-THC) - red
- Lower spot - Cannabinol – purple

9.5.6.2 Visualizes psilocyn - red which then turns blue when acidified with HCl.

9.5.6.3 Recipe: Approximately 0.5% solution of Fast Blue B salt OR Fast Blue BB salt in water.

9.5.7 Fluorescamine (Fluram^R)

9.5.7.1 Visualizes amino acids, primary amines and amino sugars.

9.5.7.2 Recipe: 20 mg Fluram^R in 100 mL acetone.

9.5.7.3 Procedure: Heat plate after spraying with reagent, then check under long wave UV light (amphetamine fluoresces green-yellow).

9.5.8 Furfuraldehyde and HCl (9.8.1)

9.5.8.1 Good for meprobamate and other carbamates.

9.5.8.2 Recipe: 10% solution of furfuraldehyde in ethanol. Overspray with concentrated HCl.

9.5.8.3 Procedure: Spray plate and heat, if necessary. Spots are black on a white background.

9.5.9 6N HCl

9.5.9.1 Used to acidify plates

9.5.10 Iodine Vapors (9.8.1)

9.5.10.1 Good for general unknowns and compounds which are not UV active. Suitable for GHB, 1,4-butanediol and GBL analysis. This is a good method of visualization if further testing is to be done on the sample on the plate, as it is reversible.

9.5.10.2 Procedure: Place iodine crystals in an enclosed chamber. Let TLC plate develop in the chamber. Many organic compounds will produce a brown spot.

9.5.10.3 Results:

- GHB – white spot on yellow background
- GBL, 1,4-butanediol – brown spot on yellow background

9.5.11 Iodoplatinate (9.8.1)

9.5.11.1 Most useful reagent for nitrogen containing compounds, may be acidified with HCl to intensify some reactions.

9.5.11.2 Recipes:

- 5 mL of 10% platinic chloride aqueous solution and 10 g of potassium iodide in 500 mL of H_2O.
- 1 g of platinic chloride and 10 g of potassium iodide in 500 mL H_2O.

9.5.11.3 If acidified iodoplatinate is preferred, either overspray the TLC plate with 6 N HCl or prepare stock solution with approximately 5% HCl.

9.5.11.4 Results may be intensified with an overspray of ceric sulfate reagent.

9.5.12 Mercuric Sulfate (9.8.8)

9.5.12.1 Used for barbiturates, which appear as white spots on off-white background. The plate may need to be sprayed heavily.

9.5.12.2 Recipe: Suspend 5 g HgO in 100 mL of H_2O. Add 20 mL concentrated H_2SO_4. Cool, dilute with 250 mL H_2O.

9.5.12.3 Mercuric Sulfate can also be freshly mixed 50/50 with the diphenylcarbazone reagent.

9.5.13 Ninhydrin (9.8.1)

9.5.13.1 Visualizes amino acid, amines and amine sugars. (9.8.1)

9.5.13.2 Recipe: Add 0.5 gram of ninhydrin to 10 mL concentrated HCl. Dilute to 100 mL with acetone.

9.5.13.3 Procedure: Spray with ninhydrin solution then irradiate the plate under long wave UV light (366 nm) for 2 minutes. Heat the plate in an approximately 100°C oven for 2 minutes.

9.5.13.4 Results: yields pink-violet spots.

9.5.14 Potassium Permanganate (9.8.1)

9.5.14.1 Visualizes unsaturated hydrocarbons. $KMnO_4$ is a good alternative to Mercuric Sulfate for barbiturates which contain a double bond. $KMnO_4$ may be used as an underspray or overspray with Iodoplatinate.

9.5.14.2 Recipe: Dissolve 1 g $KMnO_4$ in 100 mL H_2O.

9.5.14.3 Results: yields a yellow spot on a purple background.

9.5.15 Sulfuric Acid/Ethanol Reagent for Steroids (9.8.5)

9.5.15.1 Recipe: Add gradually 10 mL of conc. sulfuric acid to 90 mL of ethanol.

9.5.15.2 Procedure: Spray plate and heat gently on a hot plate to develop.

9.5.15.3 Results:

- Testosterone - green
- Testosterone esters – purple
- Oxymethalone – red
- Nandrolone decanoate – purple

9.6 Preparative Thin Layer Chromatography:

9.6.1 Introduction

9.6.1.1 Frequently, samples contain other organic compounds which interfere with the drug analysis (e.g., heroin and quinine). Preparative TLC can be used to clean up a sample for other methods of testing such as IR or MS.

9.6.1.2 If cleaning up cocaine for a base determination, be careful to use a neutral bath to develop the plate so that the original salt form will not be altered.

9.6.2 Materials

9.6.2.1 Thin layer plates

9.6.2.1.1 A section of 250μm thin layer plate can be used if only a small quantity of pure compound is needed.

9.6.2.1.2 For larger quantities, use a 1000μm plate.

9.6.3 Procedure

9.6.3.1 The sample is dissolved in an appropriate solvent and streaked along the bottom of the plate using a capillary tube, long tipped Pasteur pipette or a commercial streaking device (if available).

9.6.3.2 A standard may be spotted separately at either the beginning or end of the plate in order to identify the desired compound after development.

9.6.3.3 Develop the plate as in regular TLC.

9.6.3.4 After drying the plate, the desired area is located and marked under UV light. (For compounds not UV visible, iodine vapors can be used.)

9.6.3.5 Scrape off the desired area, wash thoroughly with solvent in a small beaker and filter to remove the silica gel. Smaller quantities can be filtered using a disposable Pasteur pipette with a pre-washed glass wool plug.

9.6.3.5.1 After development, most compounds adhere strongly to the deactivated silica gel and therefore must be washed with a fairly polar solvent. Methanol is recommended. For some compounds, an extraction from an aqueous acidic or basic solution may be necessary.

9.6.3.6 If using two-dimensional TLC, first develop the plate as usual, and then develop the plate in a polar solvent system at a 90 degree angle in order to concentrate the sample into a tighter spot. The standard would need to be removed by breaking off the portion of the plate containing the standard prior to this step. The compound is then removed from the silica as described above in Section 9.6.3.5.

9.7 Comparative Semi-Quantitative Thin Layer Chromatography:

9.7.1 Thin layer chromatography can be used to determine relative concentration between a sample and a standard. This is useful when it is necessary to determine whether a pharmaceutical preparation has been diluted or substituted. In cases where an exact assay is needed, a suitable quantitation should be performed.

9.7.2 Procedure:

9.7.2.1 Obtain or prepare a standard at the concentration expected for the sample.

9.7.2.2 Apply equal amounts of the standard solution and the sample solution to the TLC plate.

9.7.2.3 Develop and visualize the plate as described above for regular TLC.

9.7.2.4 Visually compare the size and color of the spots to determine if the substance has been substituted or diluted.

9.7.2.5 Approximate concentrations can be estimated by bracketing the observed sample concentration within appropriate standard dilutions. Visually compare the sample response to that of the closest standard dilution. This approximation should be recorded in the case notes but not indicated on the report.

9.7.3 Reporting: Any controlled substance present will be initially identified in the usual manner. The concentration or substitution of the sample will normally be addressed in the report with one of the two following statements for suspected tampering cases.

9.7.3.1 Meets label specifications.

9.7.3.2 Does not meet label specifications.

9.7.3.3 Any pharmaceutical substitutions or adulterants, properly identified, should be reported as usual. Their concentrations will not normally be required. (e.g., Diazepam substituted into a device labeled to contain morphine would be identified and reported with no concentration value required).

9.8 References:

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9.8.4 Rasmussen, K. E. and Knutsen, P. "Techniques for the detection and identification of amphetamines and amphetamine-like substances" *Bulletin on Narcotics*, Vol. XXXVII, No. 1, 1985, pp. 95-112.

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9.8.7 Sperling, Albert, "Thin-Layer and Gas Chromatographic Idenitfication of LSD", *Journal of Chromromatographic Science*, Vol. 12, May 1974, pp. 265 – 266.

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9.8.10 Macek, Karel, *Pharmaceutical Applications of Thin Layer and Paper Chromatography*, Elsevier Publishing Co. New York, 1972, p. 654.

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